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"Daily 1α-OH-D_2 in Hormone Refractory Prostate Cancer: Assessment of Clinical and Biochemical Effects"

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13. ABSTRACT *(Maximum 200 Words)*

The goals of this proposal were to examine the clinical effectiveness of vitamin D analog therapy (1α hydroxyvitamin D_2) in patients with advanced androgen-independent prostate cancer. We performed a phase II study of 1α hydroxyvitamin D_2 administered daily at doses from 12.5 to 5.0 ug/day and examined various laboratory correlative studies for any correlation with outcome. Daily administration of 1α hydroxyvitamin D_2 was well tolerated with occasional grade 1 and rare grade 2 hypercalcemia. No objective tumor responses were observed but 6 out of 20 evaluable patients had stable disease for $\geq$ 6 months. Treatment with 1 α hydroxyvitamin D_2 was associated with decreased serum PTH levels and increased urine calcium levels. Plasma TGFβ1 levels and T cell receptor ζ chain analysis in peripheral T cells did not correlate with clinical outcome as related to vitamin D-based therapy. These findings support further study of the potential clinical effectiveness of vitamin D-based therapies in advanced prostate cancer.

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INTRODUCTION

Prostate cancer is the most commonly diagnosed solid tumor among men in the United States (1). Unfortunately, thousands of men each year are diagnosed with disseminated prostate cancer which is incurable. The most effective systemic therapy for advanced prostate cancer remains androgen hormone manipulation. A better understanding of the prohormone vitamin D and the effects of its active metabolites on many different cell types has led to an exploration of its possible role in cancer prevention and therapy. Cholecalciferol, an active metabolite of vitamin D, undergoes ligand binding to a specific intracellular steroid receptor (vitamin D receptor), which via effects on DNA transcription leads to many different cellular effects (2). Preclinical study of vitamin D observed prostate carcinoma cells to be responsive to its growth inhibitory effects (3). Vitamin D was found to have significant antitumor effects against prostate cancer cells in vitro and in vivo(4). Unfortunately, clinical testing of vitamin D revealed dose-limiting calcemic effects which precluded achieving concentration exposures capable of growth inhibitory effects (5). Vitamin D analogs have been developed which maintain the same potent growth inhibitory effects with significantly less calcemic effects than vitamin D (6). 1α hydroxyvitamin D_2 is such an analog. Early phase I testing revealed less calcemic effects than vitamin D and objective tumor shrinkage in two patients with hormone-refractory prostate cancer. Therefore, we proposed a phase II study of daily 1α hydroxyvitamin D_2 in patients with hormone-refractory prostate cancer with correlative studies to assess for potential biochemical parameters of vitamin D-based clinical effect.

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BODY

The goal of task 1 is the performance of an initial phase II study of the vitamin D analog 1α hydroxyvitamin D_2 (1α-OH-D_2, doxercalciferol) in patients with advanced androgen-independent prostate carcinoma. Preliminary research had showed 1α-OH-D_2 was rapidly metabolized into two active metabolites, 1α,25(OH)$_2$$D_2$ and 1α,24(OH)$_2$$D_2$ (6). The two metabolites have similar potency to cholecalciferol (1α,25(OH)$_2$$D_3$) in inhibiting proliferation of normal and malignant cells but with considerably less calcemic effects (6). Additional preclinica work done at the UW had observed not only significant antitumor effects of the active metabolites of 1α-OH-D_2 against prostate carcinoma cells, but also increased expression of prostate specific antigen (PSA) in LNCaP cells.

Prior to clinical anticancer testing 1α-OH-D_2 had undergone extensive testing in female subjects with postmenopausal osteoporosis and male and female subjects with end-stage renal disease and secondary hyperparathyroidism (7). The safety of chronically administered 1α-OH-D_2 in these subjects and the clinical potential of vitamin D analogs in prostate cancer led us to pursue phase I study of 1α-OH-D_2 in hormone-refractory prostate cancer patients. Twenty-five (21 evaluable) patients were entered onstudy at 5 dose levels (5, 7.5, 10, 12.5, 15 ug/day). One of four patients at level 4 (12.5 ug/day) developed reversible grade 2 hypercalcemia. At level 5 (15 ug/day) in 11 evaluable patients the following toxicity was observed: 3 episodes of reversible grade 1 hypercalcemia; 1 episode each of reversible grade 2 and 3 hypercalcemia accompanied by grade 2 increases in creatinine. No other significant toxicity was observed. Objective tumor shrinkage was noted in 1 patient each at the 5 and 7.5 ug/day dose levels. Serum PSA values did not correlate with tumor responsiveness. These data led us to propose a phase II dose of 12.5 ug/day of 1α-OH-D_2.

Unlike other phase II studies evaluating new agents in prostate cancer, we did not chose either tumor shrinkage (objective tumor response) or PSA reduction as the primary endpoint or determinant of clinical benefit. The reason for not choosing PSA is related to the observation of vitamin D-induced expression of PSA even under conditions of vitamin D-induced growth inhibition. The initial evaluation of new agents in prostate cancer and other tumors has been based on the ability of cytotoxic agents to induce tumor shrinkage. Thus tumor shrinkage or response was the surrogate marker for clinical benefit. This may or may not be a reasonable surrogate for cytostatic agents which require chronic administration. It was possible, given the potential mechanism(s) of action of doxercalciferol, that a clinically beneficial effect may manifest itself only as a static state or stable disease in some patients. Thus it did not seem prudent to base the future usefulness of a novel therapy on potentially the wrong surrogate marker. Therefore, we proposed to use a 6 month stable disease (lack of progression) rate as the primary outcome measure. The benchmark level for potential clinical usefulness was to be a 40% or greater 6 month stable disease rate. The maximum trial size was set at 40 evaluable patients with an initial evaluation set at 20 evaluable patients prior to full enrollment. If 5 or more of the

first 20 patients reach 6 months with stable disease or 2 or more exhibit an objective tumor response, additional patients will be added to the maximum.

Doxercalciferol was administered orally once a day prior to the first meal of the day. Patients remained on the drug until evidence of desease progression, irreversible or life-threatening toxicity, patient refusal, or significant clinical deterioration rendering further therapy unacceptable. No attempt was made to ameliorate the calcemic effects with dietary manipulations or other medications. Disease progression was defined as the appearance of new lesions on bone scan or other radiologic studies, > 25% increase in bidimensionally measurable disease, or symptomatic worsening. All patients were entered at the 12.5 ug/day dose. Patients remained at this dose unless, grade 1 hypercalcemia (corrected for serum albumin), $\geq$ grade 2 creatinine or $\geq$ grade 3 other toxicity occurring. If the above toxicity was observed, drug was stopped for $\geq$ 1 week or until toxicity resolved and patients could be retreated at the next lower dose (10, 7.5, or 5 ug/day). Planned clinical evaluations were as follows: weekly – serum calcium, phosphorous, creatinine; monthly – 24-hour urine calcium, serum PTH, complete blood count, chemistry panel, and serum PSA; every 12 weeks – disease evaluation.

Twenty-six patients have been entered on study with 20 evaluable. Patient characteristics are as follows: median age – 70, range 57-80 years old, performance status (ECOG) was six with 0, nineteen with 1, and one with 2. Currently 6 of 20 have had stable disease for $\geq$ 6 months. There are currently 3 patients still on study, one at > 6 months, and two at > 5 months all with stable disease. There have been no observed objective responses. Patient accrual has been on hold since January 2001 waiting to assess the first 20 evaluable patients. Duration on study has varied from 1 month to 11 months. Serum PSA values have routinely increased in all patients on study. Evaluation of the initial 17 patients with progressive disease, revealed the change in PSA from onstudy to progressive disease with the majority having a 200-400% increase, 5 cases with < 200%, 1 case > 400%, and 1 case < 100% (n = 17, mean±S.D., 280±190%, range 85-960%). In patients on study > 6 months the PSA gradually increased throughout the study treatment. Since there were no objective responses, no correlation could be made with PSA.

Toxicity has been minimal to mild with one occurrence each of grade 2 and 3 hypercalcemia at 12.5 ug/day and frequent episodes of grade 1 hypercalcemia. Out of approximately 90 courses of therapy, 3 episodes of grade 1 creatinine and 1 episode of grade 2 creatinine have been observed. No other significant toxicity has been noted.

As expected serum PTH values declined and urine calcium levels increased with doxercalciferol treatment. Preliminary analysis was as follows: mean±S.D., n = 9, serum PTH (pg/ml) baseline – 32.7±11.7, day 28 – 15.3±7.1, day 56 – 10.3±7.4; Urine calcium (mg/24-hour collection) baseline – 136.3±104.5, day 28 – 196.5±196, day 56 – 427.8±176.8.

The next aim of this project was to examine other biological markers for their potential as predictors of a positive or negative clinical effect from daily administration of doxercalciferol. It was discussed earlier that using serum PSA

as an early predictor of effect has potential specific interactions with vitamin D-based effects to limit its usefulness. Thus, two experimental approaches were explored in this context because of their potential as early markers of effect but also due their potential to help elucidate vitamin D-based mechanisms of action. TGFβ's are multifunctional growth factors which have been shown to be potent negative growth factors and to be inducible by multiple novel agents (8). Evaluation of plasma TGFβ1 levels could provide early evidence of a beneficial effect as well as give insight into mechanistic issues. Whole blood was obtained weekly for the initial month, biweekly for the second month, then monthly thereafter while patients were on study. Preliminary analyses do not reveal any correlation to clinical outcome other than a slight trend toward increased values with progressive disease. Current analyzed data is as follows: n = 12, mean±S.D. ng/ml (range) baseline – 4.6±4.1 (1.7-13.3), week 1 – 6.1±5.1 (2.2-12.3), week 4 – 5.9±4.4 (2.1-16.0). At this point in the data analysis it does not appear that plasma TGFβ1 determinations will provide insight into either possible mechanistic issues or be an early predictor of clinical effect.

Researchers have found an association between neoplastic progression and suppression of endogenous immune response. An example of this is reported molecular alterations in signal transduction in T cells from mice bearing long-term tumors and in peripheral blood lyphocytes (PBLs) from patients with various malignancies. A predominant alteration is decreased expression of the T cell receptor (TCR) ζ chain which has an adverse effect on the immune response since the cytoplasmic domain of the CD3 ζ chain subunit is directly required for the signal transduction and subsequent activation of T and NK cells. Early research has observed a strong correlation between decreased TCR ζ chain expression and progressive cancers including prostate cancer (9). Evaluating TCR ζ chain expression could also provide mechanistic information as well. Vitamin D and probably its analogs have a multitude of effects on immune function.

Whole blood was obtained monthly and PBLs separated. On the day of assay cells were thawed and stained with surface monoclonal antibodies. Cells were prepared and stained with antibody to TCR ζ chain. Preliminary data is currently available. Patients on the study presented with altered subset distributions, principally a decreased percentage of T cells and an increased number of NK cells as compared to normal donors. This distribution did not change significantly during therapy. A majority of the early patients had an initial decrease in TCR ζ chain expression followed by transient rebound in TCR ζ chain expression at 2 months. Values at presentation with comparison to normal volunteers is as follows:

	Phenotype - % lymphocytes			TCR ζ Chain Expression		
	T cells %	NK cells %	NK-T cells %	T cells	NK cells	NK-T cells

normal	72.54	17.15	2.86	0.97	1.08	1.09
patients	54.19	20.69	8.98	1.38	1.13	1.52

KEY RESEARCH ACCOMPLISHMENTS

Clinical assessment of a vitamin D analog in patients with advanced androgen-independent prostate carcinoma: the initial assessment of a vitamin D analog in prostate cancer has been completed.
- The data reveals vitamin D analogs can be safely given to patients with advanced prostate cancer and there is encouraging evidence of clinical activity.
- The preliminary analysis of the first 20 evaluable patients concludes the primary goal of achieving a $\geq 40\%$ incidence of stable disease for > 6 months on therapy is still achievable, but not proven.

Assessment of the main laboratory correlative studies:
- preliminary analysis of plasma TGFβ1 levels and TCR ζ chain expression in PBLs does not reveal any correlation with clinical outcome, but further analyses are needed due to active patients on study.
- Plasma TGFβ1 levels show modest intrapatient variability and no definite trends relative to doxercalciferol treatment.
- Assessment of TCR ζ chain expression does reveal differences as compared to normal volunteers.
- Some trends in expression relative to the duration of doxercalciferol treatment have been observed but the significance of these is uncertain at this early point in the analyses.

REPORTABLE OUTCOMES

Manuscripts and abstracts

Bailey, H.H., Ripple, G., Bruskewitz, R., Bishop, C., and Wilding, G. Phase I and II trials of 1α-hydroxyvitamin D_2 in patients with metastatic hormone-refractory prostate cancer. Clin Cancer Res. 5 (suppl): 39, 1999.

Schell, K., Dresen, A., Pomplun, M.L., Tutsch, K.D., Wilding, G., and Bailey, H.H., Characteristics of TCR-ζ chain expression in lymphocyte subsets of patients with androgen-independent prostate cancer in a phase II clinical trial with the vitamin D analog, 1α-OH-D_2. Proceedings of AACR 41:278, 2000

Bailey, HH, Ripple, G, Horvath, D, Staab, MJ, Alberti, D, Arzoomanian, RZ, Miller, K, Bruskewitz, R, Bishop, C, Nunchuck, S, Wilding, G. Phase I and II trials of 1α-hydroxyvitamin D_2 in patients with metastatic hormone-refractory prostate cancer. Clinical Cancer Res 6 (11, suppl):112, 2000.

CONCLUSIONS

The primary goal of this project was to perform the first phase II study of a vitamin D analog in advanced prostate cancer. The past history of success of hormonal therapy in many types of cancer, including prostate cancer, has led to exploration of new avenues of hormonal therapy. Vitamin D-based therapy and prevention of cancer is such an approach. The preliminary study of doxercalciferol in prostate cancer patients clearly shows the safety and implies that significant clinical benefit may be derived from this approach.

This initial phase II study took a conservative approach to the dosing of doxercalciferol. It was decided to consider grade 1 hypercalcemia unacceptable. While it seemed prudent given the potential renal toxicity associated with > grade 1 hypercalcemia, it ended up limiting the dosing of doxercalciferol in this study. Every time grade 1 hypercalcemia occurred, it resulted in stopping drug and a mandatory dose reduction for the patient. All patients started at 12.5 ug/day of doxercalciferol, but very few patients remained at that dose their entire time on study. Could dosing or dose intensity of doxercalciferol influence its ability to growth inhibit prostate carcinoma? The preclinical data doesn't directly address this issue, but most studies utilized sustained dosing at the highest dose/concentration allowable. Potentially supporting the concept of some relative dose response with doxercalciferol is our own data. In examining the first 20 patients, the group can be divided into two groups of 10. Group 1 received the 12.5 ug/day dose of doxercalciferol for < 4 weeks before a treatment interruption and dose reduction, while group 2 received 12.5 ug/day for ≥ 4 weeks without interruption before any treatment interruption or dose reduction. Duration on study (in months) with stable disease is as follows: mean±S.D. (range) Group 1, n = 10, 12±9 (5-36); Group 2, n = 10, 18±11 (6-44) (p=0.19, student's t test).

The current clinical results of ≥ 6 out of 20 patients with stable disease > 6 months and the conservative dosing support continued clinical study of doxercalciferol at a similar or more aggressive dosing schedule. Potential ways of having a more aggressive dosing schema is as follows: 1) simply allowing grade 1 hypercalcemia; 2) do not reduce dose for unacceptable hypercalcemia but instead stop drug until resolvement and restart at the same dose with a planned drug holiday at a similar to slightly shorter interval; 3) dietary manipulation of calcium intake; 4) use of calcium lowering agents, e.g. bisphosphonates; 5) increased daily dosing but with more frequent drug holidays, e.g. one week on, then one week off therapy. All of these maneuvers are being tested in preclinical or clinical models with vitamin D and various analogs.

Our initial plans are to seek additional funding for continued testing of doxercalciferol in patients with hormone-refractory prostate cancer, at a more aggressive dosing schedule. Most likely pursuing a course of allowing grade 1 hypercalcemia and stopping drug (but not dose reducing at re-initiation) until > grade 1 hypercalemia resolves.

Assessment of potential laboratory-based surrogate markers for vitamin D-based beneficial effect has not revealed definite evidence that either plasma

TGFβ1 or TCR ζ chain expression can accomplish this function. It is premature to make definitive statements regarding these parameters since data analysis is not complete. But if there is little evidence to support their roles as potential markers of effect after complete analysis of the first 20 evaluable patients it will be secondary to one of the following. 1) They do not accurately reflect the tumor environment under conditions of vitamin D-based therapy; or 2) doxercalciferol therapy directly benefited very few to no patients making it impossible to detect any differences between patients benefiting or not benefiting. We will plan on continuing our current analyses and then make a decision whether to continue evaluating these parameters with future trials. An important parameter we are continuing to explore with the hope of including with future trials is assessment of VDR status in prostate carcinoma samples. Performance on VDR by ELISA on fresh samples is currently being performed. But, we hope to validate immunohistochemical analysis of archived samples. Currently newer VDR antibodies are being tested since most current antibodies to VDR suffer from cross-reactivity with other proteins of interest, e.g. heat shock proteins.

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Publications and meeting abstracts resulting from this research effort include:

Bailey, H.H., Ripple, G., Bruskewitz, R., Bishop, C., and Wilding, G. Phase I and II trials of 1α-hydroxyvitamin D_2 in patients with metastatic hormone-refractory prostate cancer. Clin Cancer Res. 5 (suppl): 39, 1999.

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